

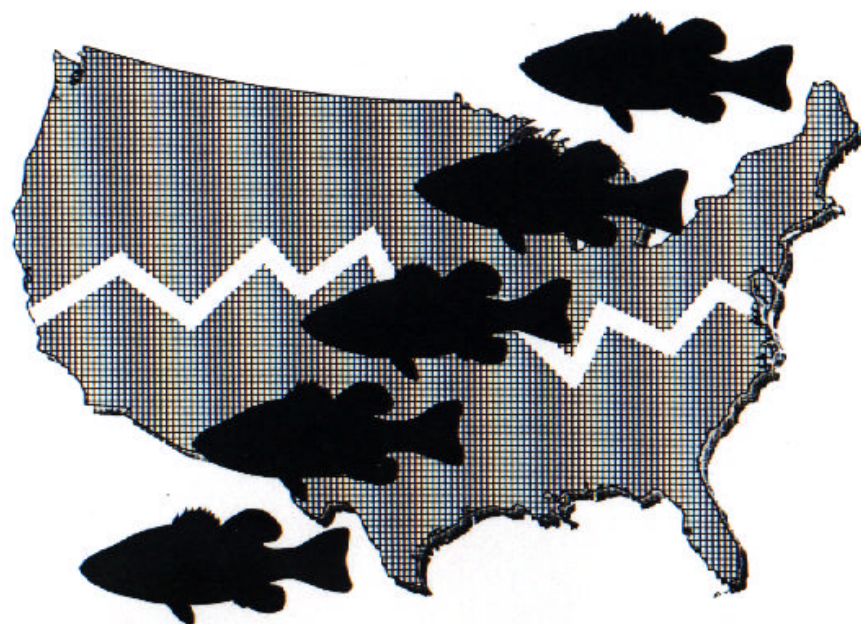


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Washington, DC 20460

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# Quality Assurance Report for the National Study of Chemical Residues in Lake Fish Tissue: Year 1 Analytical Data



*Prepared for:*

U.S. Environmental Protection Agency  
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## Chapter 1 Introduction

This report documents the quality of data gathered during the *National Study of Chemical Residues in Lake Fish Tissue* (hereafter referred to as either the “National Fish Tissue Study” or, more simply, “the study”). For reference purposes, this report provides a brief overview of the study and outlines the primary participants and the analytical parameters measured. Additional details concerning the design and implementation of this study can be found in the references listed at the end of this Chapter.

### DESCRIPTION OF STUDY OBJECTIVES AND STUDY DESIGN

The *National Study of Chemical Residues in Lake Fish Tissue* is a screening-level study designed to estimate the national distribution of selected persistent, bioaccumulative, and toxic (PBT) chemicals in fish tissue from lakes and reservoirs of the continental United States. The study involves the collection of predator and bottom-dwelling fish from 500 randomly selected lakes and reservoirs of the continental United States (excluding the Great Lakes) over a period of 4 years (~125 lakes per year). Although the study was to begin during the summer of 1999, full implementation did not commence until 2000. For this reason, samples collected during the 1999 mobilization and 2000 implementation periods cumulatively represent Year 1 of the Study.

The study design resulted from a comprehensive planning effort that included a national workshop involving more than 50 scientists from state, federal, and tribal agencies to obtain technical input on sampling design, target analytes, sampling methods and data management. The final study design is described in Reference 1 of this chapter and highlighted in Exhibit 1-1.

Implementation of the study is a collaborative effort being led by EPA’s Office of Science and Technology, within the Office of Water, with extensive support from each participating state, each EPA Region, and ORD’s Environmental Monitoring and Assessment Program (EMAP). States, tribes, and EPA Regional staff are collecting most of the fish for the study. Contractor support is being provided by Tetra Tech (for study design, orientation/training, workshops, and field sampling activities), DynCorp Environmental (for sample coordination, tracking, data review, and database development), and the following laboratories: Axyx Analytical Services in Sydney, British Columbia, Canada; Battelle Ocean Sciences in Duxbury, MA; Battelle Marine Sciences in Sequim, MA; and Pacific Analytical Inc., in Carlsbad, CA.

It should be noted that this report focuses on *data* collected during the Year 1 study period (i.e., Year 1 results) rather than on the suite of lakes statistically sampled to represent Year 1 (i.e., Year 1 lakes). Although the distinction may be confusing, it is important. The pool of 500 lakes targeted in the study was statistically sampled to meet study objectives. To ensure that study objectives could be met in the event of unforeseen circumstances, the 500 lakes were statistically subsampled and classified as Year 1, Year 2, Year 3, and Year 4. However, because these statistical designations did not always conform with available resources at the state level, some states requested and received permission to postpone collection of “Year 1 lakes” until a later year or to collect latter year lakes during the Year 1 timeframe. To avoid confusion caused by this distinction, the statistical years will be referred to as “panels” (i.e., Panel 1, Panel 2, Panel 3 and Panel 4 lakes) and the remainder of this report will focus on data gathered during the Year 1 study period. Because the Year 1 results do not directly match the Panel 1 lakes, statistical assessments of mean distributions cannot be made at this time. During the Year 1 study period, a total of 143 lakes were sampled. Ninety-two (92) of these were Panel 1 lakes, 37 were Panel 2 lakes, 6 were Panel 3 lakes, and 8 were from Panel 4.

## **Exhibit 1-1 Study Design Highlights**

### **Objective**

Estimate the national distribution of persistent, bioaccumulative, and toxic (PBT) chemicals in fish tissue from lakes and reservoirs of the continental U.S.

### **Sample Sources**

500 randomly selected lakes and reservoirs (each defined as a permanent body of water having at least one hectare in surface area with a minimum of 1,000 m<sup>2</sup> of open (unvegetated) water, a minimum depth of one meter, and a permanent fish population)

### **Sample types**

- Edible tissue (skin on fillet) composites of targeted predator species
- Total body tissue (whole fish) composites of targeted bottom-dwelling species

### **Composite definition**

Five individual fish of the same species that:

- Satisfy legal requirements of harvestable size or weight (or are consumable if no legal harvest requirements are in effect)
- Are of similar size so that the smallest individual within the composite is no less than 75% of the total length of the largest individual
- Are collected at the 'same' time (i.e., as close to the same time as possible but no more than 1 week apart)
- Are of adequate size to allow for analysis of target study analytes

### **Target Species**

Selected in accordance with EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis, Second Edition* (US EPA 1995), with preferences as cited in Reference 1 of this chapter.

### **Field QC**

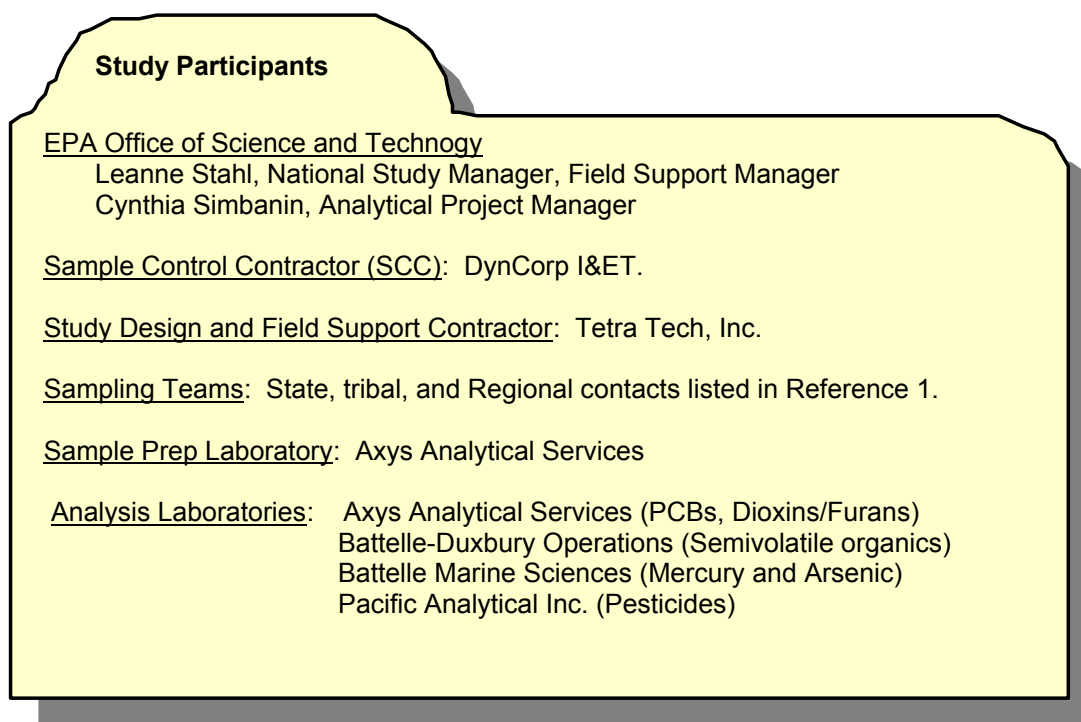
- Development and distribution of the Sample Collection Activities QAPP, field sampling plan and SOPs
- Collection of replicate fish samples on 10% of the lakes
- Use of experienced fisheries biologists to ensure use of proper procedures
- Distribution of standardized sampling kits to control contamination and ensure proper documentation
- Daily tracking and coordination of sample shipments through a centralized source
- Regional orientation/training workshops to ensure all field personnel understood objectives and design of study and to ensure consistent application of required sample collection, handling, and shipping procedures

### **Laboratory QC**

- Development and distribution of the Analytical Control and Assessment Activities QAPP
- Use of centralized Sample Prep Laboratory to minimize variability during sample grinding, homogenizing, and compositing
- Identification of quantifiable measurement quality objectives (MQOs)
- Implementation of standardized sample tracking, lab analysis, data reporting, and data review procedures
- Use of pure and traceable reference standards
- Demonstration of instrument calibration and system performance
- Periodic calibration verification
- Verification that each laboratory could achieve the required detection and quantitation levels
- Analysis of initial and ongoing QC samples to demonstrate each laboratory's ability to achieve precise and accurate results with the method
- Analysis of blanks to demonstrate the absence of contamination

## Study Participants

A detailed list of participants referenced in this report is provided in Reference 3 of this Chapter. Exhibit 1-2 below identifies the parties cited in this report.



### Exhibit 1-2. Overview of Study Participants

## References

Additional information regarding the design and implementation of this study can be found in the following references.

- (1) U.S. Environmental Protection Agency. 1999. *National Study of Chemical Residues in Lake Fish Tissue: Study Design*. USEPA, Office of Water/Office of Science and Technology, Washington, DC.
- (2) U.S. Environmental Protection Agency. 2000. *Quality Assurance Project Plan for Sample Collection Activities for a National Study of Chemical Residues in Lake Fish Tissue*. Prepared by Tetra Tech, Inc., for USEPA, Office of Water/Office of Science and Technology, Washington, DC.
- (3) U.S. Environmental Protection Agency. 2000. *Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue*. USEPA, Office of Water/Office of Science and Technology, Washington, DC.
- (4) U.S. Environmental Protection Agency. 2000. *Quality Assurance Project Plan for Analytical Control and Assessment Activities in the National Study of Chemical Residues in Lake Fish Tissue*. Prepared by DynCorp Environmental for USEPA, Office of Science and Technology, Washington, DC.

The first three documents are available from Leanne Stahl, National Study Manager, and the final document is available from Cynthia Simbanin, Analytical Project Manager at the following addresses:

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## Chapter 2

### QA Program

At the outset of the National Fish Tissue Study, EPA managers recognized that data gathered from the study would be used extensively by individuals responsible for making environmental, economic, and policy decisions. Environmental measurements always contain some level of uncertainty. Decision makers, therefore, must recognize the uncertainty associated with the data on which their decisions are based. In recognition of this, the study managers established a quality assurance (QA) program intended to ensure that data produced under the National Fish Tissue Study would meet defined standards of quality within a specified level of confidence (see Chapter 1, References 2 and 4).

Implementation of the QA Program ensured that all Measurement Quality Objectives were met in Year 1 of the Study and that not a single sample was lost or misidentified.

The study QA program prescribes minimum requirements to which all organizations that gather data are required to adhere. All of these elements were followed during Year 1, and data quality was defined, controlled, and assessed through these QA program activities. The remainder of this chapter highlights the QA program employed during the National Fish Tissue Study.

**Collaborative Study Design:** Development of the study design was a collaborative effort among EPA's Office of Water (OW), Office of Research and Development (ORD), and Office of Prevention, Pesticides, and Toxic Substances (OPPTS), with significant involvement by biologists, chemists, and statisticians in OW's Office of Science and Technology (OST), statisticians in ORD's Environmental Monitoring Assessment Program (EMAP), and chemists in OPPTS. The draft design was reviewed by experts throughout federal, state, and tribal organizations (including EPA, NOAA, USGS, and state environmental, wildlife, and fisheries management agencies) via a two-day workshop. Input obtained during this workshop was incorporated into the final study design.

**Implementation of Approved Quality Assurance Project Plans for Sampling and Analysis Activities:** Two QAPPs were developed and approved by EPA to support this study. The *Quality Assurance Project Plan for Sample Collection Activities for a National Study of Chemical Residues in Lake Fish Tissue* (May 2000) establishes data quality goals for all sample collection and handling activities and describes the QA/QC techniques that are being employed by field teams and by the field support contractor to support those goals. The *Quality Assurance Project Plan for Analytical Control and Assessment Activities in the National Study of Chemical Residues in Lake Fish Tissue* (September 2000) establishes measurement quality objectives for laboratory data that are being generated during the study and describes QA/QC techniques that are being employed by laboratory and sample control contractor staff to ensure these goals are met.

**Implementation of a Field Orientation/Training Program:** Because the study design relied on a large number of state and tribal sampling teams, EPA established a field orientation and training program to ensure that personnel responsible for sampling activities within each organization understood the study objectives, were familiar with paperwork developed specifically to document sample collection activities under the study, and were prepared to collect, document, and ship samples in accordance with the study design and the sample collection QAPP.

**Development of Study-Specific Sample Documentation and Sampling Kits:** The study design calls for collection of fish samples by multiple teams from participating states, tribes, and EPA Regions. To



ensure samples will be consistently documented by such a large and diverse group, several documentation materials have been custom-designed for the study. These forms include a:

- *Field Record Form* to document information about each lake sampled and individual specimens collected from the lake,
- *Sample Identification Label* to accompany and identify each fish specimen,
- *Chain-of-Custody form* to provide constant tracking information for all samples, and
- *Chain-of-Custody label* to seal each shipping container.

These forms are being provided annually in *custom-made sampling kits* prepared by the sample control contractor (SCC). The kits also contain contaminant-free materials needed to store each specimen (i.e., solvent-rinsed aluminum foil and food grade polyethylene tubing), a reference instruction sheet with contact phone numbers, and pre-completed forms needed to ship the specimens to the Sample Prep Laboratory for homogenization and compositing. In addition, sample *Traffic Reports* were created for use by the Sample Prep Laboratory to document each homogenized composite aliquot that is sent to either an Analysis Lab or to the Sample Repository for long-term storage. Implementation of these tools in Year 1 of the study was highly successful, with most sampling personnel completing the forms as intended.

***Daily monitoring of sampling and laboratory activities:*** To ensure effective communication among all organizations involved in the study, the field support contractor and the sample control contractor were tasked with establishing and implementing a series of procedures to prepare and distribute sampling kits, coordinate and track sample shipments, identify corrective actions in the event of lost shipments, review Field Record Forms to identify and notify EPA of fish samples that deviated from the sample criteria and recommend corrective actions, obtain laboratory analyses, review laboratory data, and generate a STORET-compatible database of study results. These activities were highly successful in controlling the quality of data during the first year of the study; not a single sample was lost during Year 1 of the study, and all potential deviations from the study design were significantly mitigated by these early identification techniques.

***Weekly to monthly reporting of project status as appropriate:*** Each of the contractors routinely reports the status of project activities to EPA so that the Study Manager can monitor study progress, notify senior EPA management of potential problems and success stories, and communicate project status to other organizations supporting the study. Such communications provide a real time means through which the study manager can notify study participants of significant issues, ranging from the need to halt sample shipments until air traffic returned to normal following the 2001 terrorist attacks, to clarifications concerning the amount of dry ice needed when shipping coolers, to the need for alternate documentation procedures to streamline the shipment of samples from the field through Customs to the Sample Prep Laboratory.

***Monthly project meetings among EPA Headquarters staff and contractors responsible for coordination of activities:*** Each month, the study manager meets with EAD staff responsible for managing laboratory and data review activities and with each team of contractors responsible for daily tracking of activities. The purpose of the meetings is to review study progress, discuss upcoming schedules, and identify and resolve problematic issues. Depending on project activities, additional staff are brought into these meetings to facilitate planning and resolution of issues. Notably, OW representatives responsible for developing and managing STORET were invited to meetings that included discussions of database designs, STORET data upload, and data distribution plans. Likewise, EAD

statisticians responsible for interpreting study results were invited to participate in meetings involving discussions of the procedures used to review, qualify, and report laboratory results.

***Implementation of thoroughly documented methods that included all QC elements needed to support quality objectives established for the study:*** A suite of EPA 1600 series methods is being employed to support the study. Each participating laboratory, including the Sample Prep Laboratory, was required to demonstrate their ability to practice these methods before preparing or analyzing samples collected in the study. Chapter 3 describes these methods in greater detail.

***Three levels of data quality assessment and application of standardized data qualifiers:*** All analytical data generated during Year 1 of the study were subjected to three levels of review. First, a pre-qualification review was performed on data submitted by each laboratory to demonstrate that the labs were qualified to prepare and/or analyze tissue samples collected during the study. Second, each submission of sample results was carefully scrutinized to verify that the samples were analyzed as directed and that supporting QC results demonstrated the quality of results generated. In evaluating these submissions, data reviewers employed a suite of standardized data qualifiers and abbreviated qualifier codes to consistently and accurately document the quality of all data generated so that both the primary data users (statisticians) in EPA Headquarters and secondary data users within states, tribes, and other organizations could make informed decisions regarding their use. A third level of data review was performed at the conclusion of the Year 1 data review process to determine if overall data quality supported study objectives. This end-of-year evaluation indicated that all measurement quality objectives were met for Year 1 of the study. Chapter 4 describes the data quality assessment procedures employed in the study.

***Implementation of standardized data format to allow all results to be reported consistently and accurately to data users:*** All data generated during the study are being compiled in a centralized, custom-developed database designed for 1) eventual upload of results to the national STORET database system, 2) statistical manipulation of results, 3) export of results to user-friendly formats such as Excel spreadsheets, and 4) consistency in data format and nomenclature across laboratories and over time.



## Chapter 3

### Analytical Methods Employed

To control variability among sample results, all samples collected during Year 1 of the study were analyzed by a single set of methods, and all analyses performed with a given method were performed by only one laboratory. Further control of variability was ensured by utilizing a single laboratory to prepare, composite, homogenize, and aliquot samples in a strictly controlled, contaminant-free environment. The methods employed by the Sample Prep Laboratory and by each analysis laboratory are described below. A complete list of the pollutants measured by these laboratories is provided at the end of this chapter in Exhibit 3-1.

#### 3.1 Sample Prep Lab Procedures

Unless otherwise directed by EPA due to deviations from the sampling design, each composite sample prepared by the Sample Prep Lab consisted of five individual fish of a single species. Bottom-dwelling species were composited as whole fish composites (i.e., the entire specimen, including the head, skin, internal organs, muscle and bones were thoroughly homogenized). Predator/gamefish were composited as skin-on (scales removed) fillet composites. All sample preparation, filleting, and homogenization activities were performed in accordance with EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories*, November 2000 (the "Fish Advisory Guidance") in a strictly controlled, contaminant-free environment. Equipment rinsates were collected during each day of sample preparation activities and analyzed to document the absence of contamination.

Upon receipt of samples from the field, the Sample Prep Lab:

- Checked that each shipping container arrived undamaged and verified that samples were still frozen and in good condition upon receipt. All samples collected in Year 1 were received frozen, but some anomalies in sample condition were reported. Fish composite samples that did not conform to established study criteria (i.e., "nonroutine" composite samples) were frozen pending a determination by EPA concerning procedures for analysis. EPA documented all processing and analysis decisions for nonroutine fish composite samples, and these decisions were entered into the study database.
- Forwarded all associated paperwork to the field support contractor for full verification of completeness and accuracy. All QA problems were successfully resolved and reported back to SCC for entry of field data into the database.
- Verified that all specimens listed on the paperwork for each composite were included in the shipment and were properly wrapped and labeled. Deviations were rare, and they were immediately reported to EPA (via SCC) for resolution and noted in the study database.
- Signed the chain of custody form and forwarded it to the Field Support Contractor with a copy to the Sample Control Contractor.
- Documented information about each specimen in a laboratory notebook.

All grinding and homogenization activities were performed in accordance with Section 7.2.2.9 of the Fish Advisory Guidance (for fillet composites) and Appendix G of the guidance (for whole fish composites) with the following exceptions:

- The lab was required to use equipment known to be free from contamination of all target analytes at the concentrations of interest.
- For predator fillets, the Sample Prep Lab used the *entire* fillet from both sides of each fish (skin-on, scales removed, with belly flap attached) instead of measuring and homogenizing equal weights of each fillet.

- For bottom feeders, each entire fish was homogenized and composited instead of compositing equal weights of individually homogenized fish.

Once homogenized and composited, the Sample Prep Lab aliquoted samples for distribution to each of the Analysis Labs. If volume allowed, the Prep Lab also prepared 'extra volume' aliquots for shipment to a sample repository for archiving. Aliquots intended for organics analyses were placed into 125-mL trace-organics clean amber jars with fluoropolymer (FEP)-lined lids. Metals aliquots were stored in 125-mL I-Chem™ Level III trace metals clean (or equivalent) glass jars, also equipped with FEP-lined lids. Each aliquot was further stored inside two food-grade plastic bags to avoid sample loss in the event of breakage. To avoid breakage, the jars were filled to no more than 80% capacity. All aliquots were frozen (-20°C) pending distribution to the Analysis Labs and the Sample Repository.

All but one Year 1 sample (a predator) yielded sufficient volume to provide each Analysis Lab with enough volume to analyze the samples either as directed by the method or by using a smaller volume. (Where possible, the extracts for the smaller volume samples were concentrated to achieve the same detection limit that could be achieved on full volume samples.) The Year 1 predator sample that did not meet volume requirements was analyzed for metals and pesticides only. The remainder of the sample was frozen pending evaluation of the corresponding bottom-feeder sample to aid in determining analytical priorities. Another sample that deviated from the study design was not analyzed pending collection of a replacement sample.

The Sample Prep Lab also was tasked with determining the lipid content of each sample. This lipid determination was performed using the procedure described in EPA Methods 1613B and 1668A, and is the same procedure used in EPA's National Dioxin Study.

The Sample Prep Lab assigned a unique five-digit EPA sample number to each composite sample and documented the sample number, and corresponding percent lipids result on a Traffic Report that accompanied each aliquot to the designated Analysis Lab. The Sample Prep Lab also prepared a series of 'blind composite duplicates' on 5% of the samples. These blind composite duplicates also were assigned five-digit EPA sample numbers and sent to the Analysis Labs in exactly the same manner as were true field sample aliquots. The blind sample aliquots were used by EPA to verify that Sample Prep Lab procedures were yielding homogeneous aliquots and to characterize variability arising from the entire sample preparation, re-distribution, and analysis processes.

### **3.2 Dioxins/Furans**

The presence and concentration of seventeen 2,3,7,8-substituted chlorinated dibenzo-*p*-dioxins and dibenzo furans (CDDs and CDFs) in each sample was determined by a slightly modified version of EPA Method 1613, Revision B (*Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS*, EPA821-B-94-005).

Modifications were made to the procedures specified in Method 1613B in order to allow for determination of dioxins and furans at levels ten times lower than those specified in the method. Specifically, the method was modified to (1) increase the tissue sample size used for analysis and (2) add a sixth calibration solution that contained all the method-specified analytes at levels lower than the levels specified in the method to verify linearity at the lower concentrations targeted.

### 3.3 Polychlorinated Bi-phenyls (PCBs)

EPA Method 1668, Revision A (*Chlorinated Biphenyls Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS*, EPA-821-R-00-002) was used to determine PCB congener concentrations in tissue samples collected during the study. There are 209 possible congeners, 12 of which have toxicological significance (i.e., the "toxic" PCBs identified by the World Health Organization). Method 1668A can unambiguously determine 126 of the 209 congeners as separate chromatographic peaks. The remaining 83 congeners do not appear as separate peaks, but elute from the gas chromatograph in groups of 2 to 6 congeners that cannot be completely resolved by the instrumentation. Ten of the 12 of the "toxic" congeners are resolved, and the remaining two congeners (PCB 156 and PCB 157) elute as a congener pair. Because PCB 156 and 157 have identical toxicity equivalency factors (TEFs), however, it is possible to accurately calculate PCB toxic equivalence (TEQ) based on the 12 toxic congeners.

For reporting purposes, each sample will be associated with 126 results that represent the 126 single PCB congeners, and another 33 results that represent co-eluting congener groups for the remaining 83 congeners, for a total of 159 PCB congener 'results'. In addition, each sample will be associated with 10 values corresponding to the 10 possible levels of chlorination for the parent biphenyl. Each of these 10 values represents the sum of the concentrations of all of the congeners in a given level of chlorination (i.e., a total of the mono-chlorinated PCBs, a total of the total di-chloro PCBs, etc). Finally, each sample is associated with a grand total PCB value, which represents the sum of the 126 congener results plus the 33 values for the co-eluting congeners. All told, states and other study partners will receive 170 unique PCB records for each sample (126+33+10+1), and 11 of these records represent totals drawn from the first 159 records (126 + 33).

### 3.3 Total Mercury

Total mercury (Hg) concentrations were determined by EPA Method 1631, Revision B (*Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*) and its Appendix (*Digestion Procedures for the Determination of Total Mercury in Tissues, Sludge, Sediments, and Soils*).

### 3.4 Arsenic Species

Total inorganic arsenic, arsenic (III), monomethylarsonic acid (MMA), and dimethylarsonic acid (DMA) were directly determined by Method 1632, Revision A (*Chemical Speciation of Arsenic in Water and Tissue using Hydride Generation Quartz Furnace Atomic Adsorption Spectrometry*). Arsenic (V) was determined by mathematically subtracting the measured concentration of arsenic (III) from the measured concentration of total inorganic arsenic. Strictly speaking, the techniques provided in Method 1632A allow for determination of the valence states of arsenate (V) and arsenite (III) rather than the species of inorganic arsenic. The actual species of inorganic arsenic are assumed to be those predicted by a geochemical equilibrium model. Total arsenic (which includes organic forms such as arsenobetaine) was not measured. Although it is commonly found in fish, arsenobetaine was not be targeted in the study because of its low toxicity to fish and humans.

### 3.5 Organochlorine Pesticides

Organochlorine pesticides and total Aroclors were determined by Method 1656, Revision A (*Organo-Halide Pesticides in Wastewater, Soil, Sludge, Sediment, and Tissue by GC/HSD*), except that sample extracts were concentrated by a factor of five beyond method-specified levels before instrumental

analysis in order to ensure that all target pesticides could be quantified at levels equal to or lower than the Screening Values published in EPA's Fish Advisory Guidance.

### **3.6 Organophosphate Pesticides**

EPA Method 1657, Revision A (*Organo-Phosphorus Pesticides in Water, Soil, and Tissue by GC/FPD*) was used to determine the presence and concentration of organophosphorus pesticides listed in Table 1.

### **3.7 Semivolatile Organics**

The remainder of the target organic pollutants were analyzed by a modified version of EPA Method 1625, Revision C (*Semi-volatile Organic Compounds by Isotope Dilution GCMS*). The modifications involved fractionating the samples by gel permeation chromatography (GPC) to yield a fraction containing the phthalates and some of the lower molecular weight lipids and a lipid-free fraction containing the polar target compounds. The phthalate/lipid fraction was further cleaned using Alumina and then recombined with the lipid-free fraction so that all target analytes could be determined in a single run.

**Exhibit 3-1**  
**National Fish Tissue Study Target Analytes and Corresponding Analysis Methods**

Analysis Method	Target Analyte	
Dioxins and Furans by Isotope Dilution High-resolution Gas Chromatography/Mass Spectrometry ( <i>Method 1613, Revision B</i> )	2,3,7,8-TCDD 2,3,7,8-TCDF 1,2,3,7,8-PeCDD 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,7,8-HxCDF	1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDD 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDD OCDF
Total Mercury by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry ( <i>Method 1631, Revision B with Appendix A - Digestion procedures for total mercury in tissue, sludge and sediment</i> )	Mercury	
Arsenic Speciation by Arsine Generation, Chromatography, and Atomic Absorption Spectrometry ( <i>Method 1632, Revision A</i> )	Monomethylarsonic acid (MMA) Dimethylarsinic acid (DMA)	Arsenic(III) Arsenic (V) Total inorganic arsenic
Polychlorinated Biphenyls by Isotope Dilution High-resolution Gas Chromatography/Mass Spectrometry ( <i>Method 1668, Revision A</i> )	159 congeners, including the following 12 "toxic" congeners: 3,3',4,4'-TeCB 3,4,4',5'-TeCB 2,3,3',4,4'-PeCB 2,3,4,4',5'-PeCB 2,3',4,4',5'-PeCB 2',3,4,4',5'-PeCB	3,3',4,4',5'-PeCB 2,3,3',4,4',5'-HxCB 2,3,3',4,4',5',5'-HxCB 2,3',4,4',5,5'-HxCB 3,3',4,4',5,5'-HxCB 2,3,3',4,4',5,5'-HpCB
Organochlorine Pesticides by Gas Chromatography ( <i>Method 1656, Revision A</i> )	4,4' -DDD (TDE) 4,4' -DDE 4,4' -DDT Aldrin cis and trans Nonachlor Dicofol Dieldrin Endosulfan sulfate Endosulfan I Endosulfan II Endrin Ethalfuralin (Sonalan) Heptachlor Heptachlor epoxide Isodrin Kepone (Chlordecone) Methoxychlor Mirex Octachlorostyrene Oxychlordane	Aroclor-1016 Aroclor-1221 Aroclor-1232 Aroclor-1242 Aroclor-1248 Aroclor-1254 Aroclor-1260 Pendamethalin (Prowl) Pentachloronitrobenzene (PCNB) cis-Permethrin trans-Permethrin Toxaphene Trifluralin $\alpha$ -BHC $\alpha$ -Chlordane (cis-Chlordane) $\beta$ -BHC $\gamma$ -BHC (Lindane) $\gamma$ -Chlordane (trans-Chlordane) $\delta$ -BHC Pentachloroanisole



Analysis Method	Target Analyte	
Organophosphorus Pesticides by Gas Chromatography (Method 1657, Revision A)	Chloropyrifos Diazinon Disulfoton Ethion Paraoxon	Parathion (ethyl) Terbufos Terbufos sulfoxide Terbufos sulfone
Semivolatile Organic Compounds by Isotope Dilution GC/MS (Method 1625, Revision C with modifications for tissue)	1,2,4,5-Tetrachlorobenzene 1,2,4-Trichlorobenzene 1,2-Dichlorobenzene 1,3-Dichlorobenzene 1,4-Dichlorobenzene 2,4,5-Trichlorophenol 3,3'-Dichlorobenzidine 4,4'-Methylenebis(2-chloroaniline) 4-Bromophenyl phenyl ether 4-Nonylphenol Acenaphthene Acenaphthylene Anthracene Benzo(k)fluoranthene Benzo[a]anthracene Benzo[a]pyrene Benzo[b]fluoranthene Benzo[g,h,i]perylene Benzo[j]fluoranthene Bis(2-ethylhexyl)phthalate 1,2,3-Trichlorobenzene* 1,2-Diphenylhydrazine* 2,2'-Difluorobiphenyl* 2,3,6-Trichlorophenol* 2,4,6-Trichlorophenol* 2,4-Dichlorophenol* 2,4-Dimethylphenol* 2,4-Dinitrophenol* 2,4-Dinitrotoluene* 2,6-Dinitrotoluene* 2-Chloronaphthalene* 2-Chlorophenol* 2-Nitrophenol* 2-Picoline* 4-Chloro-3-methylphenol* 4-Chlorophenylphenyl ether* 4-Nitrophenol* alpha-Terpineol* beta-Naphthylamine* Biphenyl* Bis(2-chloroethoxy)methane* Bis(2-chloroethyl)ether* Bis(2-Chloroisopropyl)ether* Carbazole* Di-n-octyl phthalate*	Butyl benzyl phthalate Chrysene Dibenzo[a,h] anthracene Di-n-butyl phthalate Diethylstilbestrol (DES) Fluoranthene Fluorene Hexachlorobenzene Hexachlorobutadiene Indeno[1,2,3-cd]pyrene Naphthalene Nitrobenzene Pentachlorobenzene Pentachlorophenol Perylene Phenanthrene Phenol Phenol, 2,4,6-tris(1,1-dimethylethyl)- Pyrene Tetrabromobisphenol A Di-n-propylnitrosamine* Dibenzofuran* Dibenzothiophene* Diethyl phthalate* Dimethyl phthalate* Diphenyl ether* Diphenylamine* Hexachlorocyclopentadiene* Hexachloroethane* Isophorone* n-Decane* n-Docosane* n-Dodecane* n-Eicosane* n-Hexacosane* n-Hexadecane* n-Nitrosodimethylamine* n-Nitrosodiphenylamine* n-Octadecane* n-Tetradecane* n-Triacontane* p-Cymene* 2-Methyl-4,6-Dinitrophenol* Styrene*

\*This analyte was not considered a target analyte for study purposes, but since it was listed as a target analyte in Method 1625, it was determined as a means of obtaining additional useful data for the study.

## Chapter 4

### Data Quality Assessment

Three levels of review were applied to all data generated in Year 1 of the National Fish Tissue Study. First, a pre-qualification review was performed prior to analysis of field samples to verify that each laboratory was qualified to analyze the tissue samples in accordance with the prescribed methods. Second, ongoing reviews were performed to verify that the results of each data submission were, in fact, generated in accordance with all method and study requirements. Finally, overall data quality was evaluated at the end of Year 1 to verify that data as a whole were meeting established measurement quality objectives. The procedures employed for each of these three data review levels are described in Sections 4.1 through 4.3

#### 4.1 Pre-qualification review

Prior to preparing or analyzing field samples collected during Year 1 of the study, each laboratory was required to submit data demonstrating their ability to achieve the *sensitivity*, *precision*, and *accuracy* goals established for the study.

Labs did not analyze Year 1 fish samples until they submitted pre-qualification data demonstrating they could achieve the sensitivity, precision, and accuracy goals defined for the study.

##### 4.1.1 Sensitivity

###### Sensitivity Goals

Analytical sensitivity reflects the minimum concentration of an analyte above which a data user can be reasonably confident that the analyte was reliably detected and quantified. For this study, the *method detection limit (MDL)* and the *minimum level (ML)* of quantitation were used to define the sensitivity of each measurement process.

The MDL is defined as the measured concentration at which there is 99% confidence that a given analyte is present in a given sample matrix. Prior to analyzing field samples collected in Year 1 of the study, each laboratory was required to perform an MDL study in accordance with the procedures specified by EPA at 40 CFR 136, Appendix B.

Quantitative sensitivity in this study was established by the minimum level (ML). The ML is defined as the lowest concentration at which the entire analytical system gives a recognizable signal and acceptable calibration for an analyte. The ML is equivalent to the lowest calibration standard analyzed by a specific analytical procedure, assuming that all the method-specified sample weights, volumes, and processing steps have been employed. The EPA 1600 series methods described in Chapter 3 specify MLs for tissue and/or aqueous samples. Generally speaking, MLs are roughly three times greater than the MDL and are comparable to the American Chemical Society's Limit of Quantitation (LOQ).

In accordance with study objectives, each laboratory was required to demonstrate it could achieve MLs that were equal to or lower than those listed in the analytical method they would be using in the study. The only exceptions were as follows:

- The laboratory tasked with analyzing samples for dioxins/furans was required to achieve MLs that were ten times lower than those specified in EPA Method 1613B. This was accomplished by (1) increasing the tissue sample size used for analysis to increase measurement sensitivity, and (2) adding a sixth calibration solution that contained all the method-specified analytes at levels lower than the levels specified in the method to verify linearity at the lower concentrations targeted.
- The laboratory tasked with analyzing the organochlorine pesticide samples was instructed to further concentrate its sample extracts in order to quantify all the target pesticides at levels that were equal to or lower than the Screening Values published by EPA in the Fish Advisory Guidance.
- The laboratory tasked with determining total mercury was permitted to target an ML of 2 ng/g instead of the 1 ng/g figure cited in the tissue appendix to Method 1631B. The allowed ML of 2 ng/kg was considered to be acceptable because it is well below the EPA's recommended screening value for mercury.

### Sensitivity Assessments

The Prep Lab was responsible for receiving, filleting (where appropriate), homogenizing, aliquotting, and distributing samples to the analysis laboratories. Because these processes could theoretically affect the results generated by all other laboratories, it was critical to demonstrate that Prep Lab processes would not introduce contamination of any target analytes at the levels of interest in this study. To do so, the Prep Lab was required to analyze equipment blanks (rinsates) before preparing any samples collected in Year 1 of the study. In conjunction with this, the Prep Lab was required to perform MDL studies to demonstrate the lab's ability to measure blanks at the levels of interest in the study. These Prep Lab MDL studies were performed in reagent water. Reagent water was used as a reference matrix for the equipment rinsates generated to demonstrate the absence of contamination.

The Analysis Labs were required to perform their MDL studies in a reference tissue matrix, using the same analytical methods they would be using in the study (see Exhibit 1-2). When possible, actual fish tissue was used for the MDL studies. However, it was often not possible to locate fish that were free of the analyte of interest at the low detection limits targeted in this study. In such cases, an alternative matrix, such as chicken breast or corn oil, was used.

Each MDL submission was reviewed to verify that:

- (1) The MDL procedures specified at 40 CFR 136, Appendix B were followed correctly, with respect to the number of replicates, the spiking levels, and the statistics applied in determining the MDL
- (2) The laboratory used the analytical method(s) they would be employing in the study to analyze actual tissue (or, for the Prep Lab, equipment rinsate) samples when performing their MDL studies
- (3) The laboratory performed their MDL studies on a calibrated instrument
- (4) The laboratory used an appropriate reference matrix when performing their MDL studies, and
- (5) The MDLs determined by the laboratory supported the ML that would be targeted in the study (i.e., the MDLs were at least three times lower than the target MLs)

After evaluating the MDL results and, where necessary, obtaining clarification or missing information from the laboratories, the MDL data submitted by each laboratory for metals, dioxins, PCB congeners, and pesticides were deemed to meet method and study requirements. For the semivolatile organic pollutants, all of the Year 1 MDL study results were below the method-derived MLs for tissue samples;

however, a few instances occurred in which the measured MDL was within a factor of three of this target. These cases included: n-Decane; 1,2-dichlorobenzene, 1,3-dichlorobenzene; 1,4-dichlorobenzene; p-cymene; hexachloroethane; Di-n-butylphthalate; bis(2-ethylhexyl)phthalate, and styrene. This was not considered to be a problem because 1) the instrument was calibrated at the minimum level of quantitation (ML) and 2) non-detects were reported at the ML rather than the MDL. Also, the measured MDLs for some of the pollutants such as the phthalates and n-decane were considered to be elevated by pollutant contributions from the reference matrix used, and it was evident that other pollutants, such as the dichlorobenzenes, could be detected at much lower levels (the measured MDLs were elevated by variability among the replicate measurements).

#### Reporting Thresholds for Sensitivity

As noted above, all of the 1600 series methods list MLs for aqueous and/or tissue samples. The appendices to Methods 1631B (mercury) and 1632A (arsenic) give tissue-based MDLs and MLs. These method-specified thresholds were used as reporting thresholds in the study for arsenic, but, as noted above, the laboratory-determined mercury ML of 2 ng/g was approved for use in this study. For consistency, the laboratory-determined mercury MDL also was used as the reporting threshold for detection limit sensitivity.

Method 1613B (dioxins/furans) provides MLs applicable to solids and tissues but, with the exception of 2,3,7,8-TCDD, does not provide corresponding MDLs. Because the method was modified as described in Section 3.2 to quantify dioxins/furans at levels 10 times lower than specified in the method, the quantitation limit thresholds reported in the database reflect the method-specified MLs divided by a factor of 10. Laboratory-determined MDLs were used as the reporting threshold for detection limit sensitivity. As noted above, these laboratory-determined MDLs were at least a factor of three lower than the target MLs.

Method 1668A (PCB congeners) provides estimated MDLs and MLs that were derived based on the standard deviation of single lab blank measurements. Because these levels have not been finalized, laboratory generated MDLs and MLs were used as the detection and quantitation limit thresholds, respectively. As noted above, the laboratory's calibration curve encompassed their ML, and the laboratory-determined MDLs were at least a factor of three lower than the target MLs.

Methods 1656A (organochlorine pesticides) and 1657A (organophosphate pesticides) provide recommended MLs for tissue samples and list recommended calibration standards intended to support the ML. These method-specified MLs were used as reporting thresholds for Method 1657A. For Method 1656A, the method MLs were divided by a factor of 5 to account for the five-fold extract concentration step described in Section 3.5 of this report and in the sensitivity goals discussion above. The laboratory MDLs, determined in fish tissue, were used as the threshold for reporting detection limit sensitivity. As noted above, these MDLs supported the MLs targeted in the study.

Method 1625 (semivolatile organics) does not specify minimum levels of quantitation (MLs) in tissue, nor does the method provide MDLs in either tissue or aqueous samples. Therefore, target tissue-based MDLs were mathematically derived from the method-specified aqueous MLs by converting aqueous units to solid units, accounting for the sample mass used in the tissue measurements, and dividing the resulting tissue MLs by 3. These method-derived MDLs and MLs were used as reporting limits for the semivolatile pollutants. In some cases, such as hexachlorobenzene, the method-derived MDLs were significantly higher than both the laboratory-determined MDLs and the EPA Screening Values. In such cases, it was determined that any 'hits' reported above the laboratory-derived MDL but below the method-derived MDL would be included in the database and flagged with "LJS" to indicate this occurrence. In

Year 1, none of the data received this flag because the results were either detected above the study MDL or not detected at all.

#### *4.1.2 Initial Demonstration of Precision and Accuracy*

Prior to analyzing field samples collected during Year 1 of the study, each laboratory was required to demonstrate its ability to achieve precise and accurate results with the required analytical method. To do so, laboratories were required to prepare and analyze Initial Precision and Recovery (IPR) samples as described in each method.

IPR samples consisted of four aliquots of a reference matrix spiked with a known level of the target analytes. The reference matrix was chosen to serve as an indicator of method performance that could be expected for the tissue samples collected in the study. Accuracy was measured by determining the average recovery in the replicate IPR samples; precision was measured by calculating the relative standard deviation (i.e.,  $RSD = \text{standard deviation}/\text{mean}$ ) of the measured levels in the IPR samples.

Each laboratory's IPR submission was reviewed to verify that:

- An appropriate reference matrix and spiking levels were used to prepare the four replicate samples
- The designated 1600 series method was used to analyze the samples
- The samples were generated on a properly calibrated instrument
- Calculations of analyte recovery and precision were performed correctly

After evaluating these factors and, where necessary, obtaining clarification or missing information from the laboratories, the IPR data submitted by each laboratory was deemed to meet method and study requirements and the laboratories were considered to be pre-qualified to analyze samples collected in Year 1 of the study.

## **4.2 Individual Data Package Review**

Upon completion of sample preparation activities, each sample was assigned to a "sample delivery group" (SDG) for analysis purposes. Each SDG consisted of an arbitrarily assigned group of 20 samples assembled to simplify sample distribution and minimize the number of QC samples that had to be prepared by each analytical laboratory. (Most methods specify that QC samples be prepared at a frequency of one per 20 field samples or one per analytical batch, whichever is smaller.) Laboratories also were asked to report their data by SDG to standardize the size of each data package and expedite data review processes.

All Year 1 field results and supporting QC data were carefully reviewed to determine if the method had been properly followed and if all systems were in control during sample preparation and analysis. QC deviations were assessed to determine if they had an impact on the data, and these deviations and assessments were coded directly into the study database with flags and descriptive comments.

When submitting results for each SDG, the laboratories were required to submit all results associated with analysis of the samples in the SDG. This included results of the fish tissue samples analyzed, as well as results associated with any support QC measurements (e.g., instrument calibration, blank, and spike data).

Results were to include both summary level data (the final measurement) and raw data (spectra, chromatograms, bench worksheets, etc.)

Each data package was thoroughly reviewed to ensure that:

- All samples listed on the corresponding field and prep lab documentation were analyzed and that results were provided
- Each analyte reported was uniquely identified with a Chemical Abstract Registry Service number (CAS number) to eliminate any ambiguity in naming conventions. If a CAS number was not available (e.g., for co-eluting PCB congeners), a unique Pollutant Identification Number (PIN) was created by SCC to uniquely identify each pollutant reported.
- All required QC samples were analyzed and these QC samples met specified acceptance criteria
- Results were provided for each sample analyzed, including any dilutions and reanalyses, and for all associated QC samples
- Required data reporting forms or electronic formats were provided for each of the field sample and/or associated QC analyses
- Raw data associated with each field sample and QC sample was provided with each data package, and the instrument output (emission intensity, peak height, area, or other signal intensity) was traceable from the raw data to the final result reported
- Any problems encountered and corrective actions taken were clearly documented.

If anomalies were found, the laboratory was contacted and asked to provide the missing data, clarifications, and/or explanations so that a comprehensive data review could be performed to verify the quality of their results. Results of these data reviews were documented directly in the database through the application of standardized data qualifier flags and descriptive comments concerning the reliability of the flagged results. Exhibit 4-1 at the end of this chapter summarizes flags and comments applied to the Year 1 data as a result of the review process described in subsections 4.2.1 - 4.2.7.

It is important to note that, because several of the methods used in the National Fish Tissue Study contain a large number of analytes that are being tested simultaneously, there is always a small statistical probability that QC results for some of these compounds will occasionally fail to meet method specifications. Likewise, the large number of samples collected and the complex matrices being analyzed suggest some probability of occasional QC failures. In other words, the presence of QC failures and data qualifiers in the Year 1 data set does not automatically suggest poor data quality in this study. To the contrary, EPA believes that the overall quality of data generated in this study was quite high as evidenced by the limited number of failures described in Sections 4.2.1 through 4.2.7, and by the fact that the overall dataset met all measurement quality objectives established for the study, as described in Section 4.3.

#### *4.2.1 Assessment of Instrument Calibration*

All of the methods used in the study require the laboratory to calibrate their instruments using a series of standards that covers a range of target analyte concentrations. This initial calibration provides a quantitative determination of instrument response and generates qualitative criteria for analyte identification. Methods 1631B (mercury), 1632A (arsenic), 1656A (organochlorine pesticides), and 1657A (organophosphate pesticides), require a three-point calibration (i.e., the use of three calibration standards that contain the target analytes at low, medium, and high concentrations). Methods 1625 (semivolatile organics) and 1668A (PCBs) specify the use of five standards to calibrate the instrument. Method 1613B requires the use of five standards, but as noted above in Section 4.1.1, a sixth calibration standard was used in this study to achieve lower quantitation limits.

The relationship between the response of an analytical instrument to the concentrations or amounts of an analyte introduced into the instrument is referred to as the “calibration curve”. The 1600 series methods used in the study contain specific criteria for determining the linearity of calibration curves. When the applicable criterion is met, the calibration curve is considered to be sufficiently linear to permit the laboratory to use an average response factor or calibration factor, and it is assumed that the calibration curve is a straight line that passes through the zero/zero calibration point. If the calibration curve is not linear, an alternative approach must be used to quantify sample results. This means that a regression line or other mathematical function must be employed to relate instrument response to the concentration.

Each data submission was reviewed to verify that the appropriate number of calibration standards were used and that the resulting calibration curve either met the linearity specifications in the method or that the calibration curve was used to quantify samples. *All Year 1 data were generated on instruments that met the linear calibration requirements specified in the referenced method.*

Initial calibration data submitted with each data package also were examined to verify that the calibration curve encompassed the MLs targeted in this study. The use of the ML as a point on the calibration curve is the principal means by which to assure that measurements made at the quantitation level are reliable. *All Year 1 data were generated on instruments that were properly calibrated at or below the MLs in this study.* In addition, all but one of the results reported in the study were analyzed within the instrument calibration range. This semivolatile organic result was coded with “REXC” to indicate that the result exceeded the calibration range and was further qualified as an “Estimated Value”.

Because analytical instruments are subject to drift over time, analytical methods typically require periodic analysis of standards to verify the instrument remains calibrated throughout the duration of analysis. The 1600 series methods used in the National Fish Tissue Study specify that the calibration verification be performed by analyzing a mid-point standard. The concentration of each analyte in this standard is determined using the initial calibration data and compared to the specifications in the method. If the results are within the method specifications, the laboratory is allowed to proceed with analysis without recalibrating and to continue using the initial calibration data to quantify sample results. If calibration verification results fall outside the required limits, the laboratory is required to re-calibrate their instrument before proceeding with sample analysis. The frequency of this calibration verification varies according to the instrumentation (more frequent verification is required for instruments that are highly prone to drift) and is specified in the method. Verifying calibration at the method-specified frequency allows for early identification of problems and minimizes the need to re-analyze samples that might otherwise have been analyzed on an uncalibrated instrument.

Each Year 1 data package received was reviewed to verify that:

- (1) Calibration verification was performed at the required frequency using appropriate calibration standards, and
- (2) Results of the calibration verification met method specified acceptance criteria, or
- (3) If results did not meet method specified criteria, the laboratory re-calibrated the instrument before proceeding with sample analysis.

If the calibration verification requirements were not met, the reviewer evaluated the data package to verify that the laboratory followed the corrective action dictated by the method and that results were not affected. Although more than 100,000 field sample results were generated during the first year of the study, only 6 of these results were flagged with calibration verification qualifiers. All of these were for organochlorine pesticides, an outcome that is not surprising given the large number of pesticides targeted

by the method and the fact that each pesticide is determined on two separate columns. (When the pollutant was found using both columns, the lowest of the measured results was reported since that value could be supported by both measurements.) Reported results that failed to meet the recovery specifications for Method 1656A were qualified with either "LVER" to indicate a low recovery of the calibration standard or "HVER" to indicate a higher recovery of the standard.

99.993% of the 100,018 field sample results gathered in Year 1 of the study, met all instrument calibration requirements. Of the 7 results flagged for calibration reasons, one exceeded the calibration range; the remaining 6 were pesticides confirmed on both columns, but associated with a calibration recovery that deviated from method specifications on one column.

#### 4.2.2 Reporting Thresholds

Each laboratory was instructed to report all positive results that met all method-specified criteria (i.e., 'hits') down to the MDL. The labs were further instructed to apply a "J" flag to any results reported above the MDL but below the ML.<sup>1</sup> The purpose of the "J" flag was to indicate that, although the pollutant was detected, it was detected at a value below the quantitation limit. In other words, the presence of a "J" flag suggests the reported value is qualitative (the pollutant is definitely present) but not quantitative (the reported value is an estimate of the true concentration). **Note** : The PCB lab was instructed to include these "J" flagged results when reporting congener totals for the PCBs. Because nearly all samples had at least a few "J" flagged PCB congeners, nearly all the samples also have a "J" flag on total PCB congener values.

The MDL is designed to provide a 99% level of confidence that when an analyte is reported as being present, it really is present. The converse is not true, however. If an analyte is reported as not being present at the MDL level, a 50% possibility exists that the result is a false negative. To ensure that results reported as non-detects are reliable indicators of the true concentration at which the pollutant could not be detected, the reporting threshold for non-detects was set at the ML.

Positive results were reported to the MDL and flagged with a "J" if the results were below the quantitation limit (ML). J-flagged results were further annotated as "Estimated Values" to caution users that the results were qualitative rather than quantitative.

All sample results submitted during Year 1 of the National Fish Tissue Study were carefully reviewed to verify that the laboratory adhered to these reporting conventions.

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<sup>1</sup>MDLs reported in the study database reflect the study MDLs approved during Year 1 of the study as discussed in Section 4.1.1. Because MDLs are determined in a single laboratory, measured MDL results are subject to slight changes over time and between laboratories. For pollutants, such as hexachlorobenzene and terbufos, where the recommended Screening Value was below the 'Study MDL', but above a lab-determined MDL, any results reported between the Study MDL and the lab-determined MDLs were reported in the database with a LJS (e.g., "J flagged result was between the lab and study MDLs").



#### 4.2.3 Analysis of Blanks

Blanks are used to verify the absence of contamination that may occur at any point in the measurement process. In the National Fish Tissue Study, many analytes were targeted at extremely low concentrations comparable to or lower than those typically found in the ambient environment. Therefore, frequent analysis and assessment of blanks was critical to determine if measured sample concentrations were biased by the presence of contamination during sample collection, handling, or analysis. In this study, the following blanks were used:

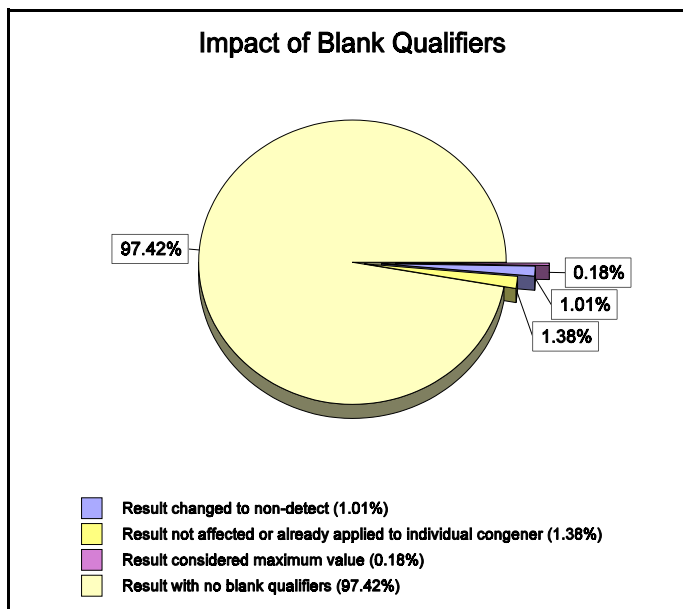
- *Equipment blanks* were used by the Sample Prep Lab to verify that the procedures and equipment used to prepare, fillet, homogenize, and or aliquot the samples were not introducing contamination. These blanks consisted of reagent water (i.e., water known to be free of the target analytes) that were run through all equipment used to process the samples in the facility where sample processing occurred. A series of blanks was prepared and analyzed before any samples were prepared in the study to verify the equipment and procedures to be used in the study were clean. The laboratory was required to reduce contamination to a level below all target MLs, and requested to reduce contamination below the target MDLs to the maximum extent possible. (It was understood that complete elimination of some ubiquitous contaminants, such as mercury and certain PCB congeners, at the low levels targeted in this study would be extremely difficult, if not impossible.) After lab cleanliness was verified, the lab continued to prepare and analyze an equipment blank on each day of sample preparation/homogenization activity.
- *Calibration blanks* were used during metals analysis to verify that contamination was not being introduced by the analytical system. These calibration blanks were to be analyzed immediately after calibration verification.
- *Method blanks* were used by each Analysis Lab to verify that contamination was not being introduced by the analytical process (i.e., the combination of the sample digestion or extraction procedures and the analytical system).

All equipment blank results submitted by the Sample Prep Lab were evaluated to verify that 1) the equipment blanks were free of contamination below the MLs targeted in this study, and 2) that any contamination reported below the ML but above the MDL was considered when evaluating corresponding tissue sample results. To evaluate the potential contribution of equipment contamination to corresponding tissue samples, the mass of contaminant detected in the equipment blank was determined by multiplying the concentration of the contaminant reported in the equipment blank by the volume of rinse water used to generate the blank. The worst-case concentration of the contaminant in each corresponding tissue composite was calculated by dividing the mass of the contaminant in the equipment blank by the total tissue mass of each composite. This calculation assumes that any equipment-related contamination in the composite sample would be equally distributed through the sample by the compositing process. The effects of equipment blank contamination were then assessed as follows:

- If the analyte was detected in the equipment blank but was not detected in the associated tissue samples, the sample data were considered to be acceptable. Such tissue data were qualified with “B” and “RNAF” to indicate that that equipment blank contamination was present but the sample result was not affected by it.
- If the analyte was detected in the associated tissue samples at levels far greater (i.e., at least ten times more) than the levels detected in the equipment blank, the effect of the blank was considered to be negligible and the tissue sample data were considered to be acceptable. Such data were qualified with “B” and “RNAF” to indicate that equipment blank contamination was present but the sample result was not affected by it.

- If the analyte was detected in the tissue samples at levels close to (i.e., within 5 times) the level detected in the equipment blank, there is no means by which to ascertain if the tissue result reported was due to contamination. In such cases, the result reported by the lab was changed to a non-detect at the ML and coded with “B” and “RNON” qualifiers to indicate the change.
- If the analyte detected in the tissue sample was more than 5 times but less than 10 times the concentration detected in the equipment blank, the tissue sample result was coded with “B” and “RMAX” qualifiers to indicate a possible high bias from contamination.

Blanks submitted by each analysis lab were reviewed and flagged according to the same approach. In applying these rules, data reviewers were careful to consider the impact of dilution on the sample results (i.e., the concentration of a diluted sample was compared to the blank result multiplied by the dilution factor that was applied to the sample). For example, if 12 ppb of contaminant was found in the blank, and the associated sample extract was diluted by a factor of 6 relative to the extract from the blank, then the sample result would have to be greater than  $12 \times 6 \times 10$ , or 720 ppb to be considered acceptable. (The result times the dilution factor times the 10 times rule described above.) If the sample result was reported to be between 360 ppb and 720 ppb, it would be flagged with “B” and “RMAX” qualifiers to indicate a possible high bias resulting from contamination as dictated by the between 5 times and 10 times rule described above.



In all, approximately 3,600 of the 100,000 tissue sample results generated during Year 1 of the study were qualified due to blank contamination, and more than 2,800 of these were these qualifiers were applied to non-toxic PCB congeners which are ubiquitous in the environment and very difficult to eliminate at the low levels targeted in this study. In addition, most of the qualifiers were used to indicate that the results were determined to be not affected by the blank contamination (i.e., flagged with "B, RNAF") or were duplicatively applied as a result of PCB congener summation (i.e., if an individual congener received a blank qualifier flag, the qualifier also was applied to the corresponding congener total.)

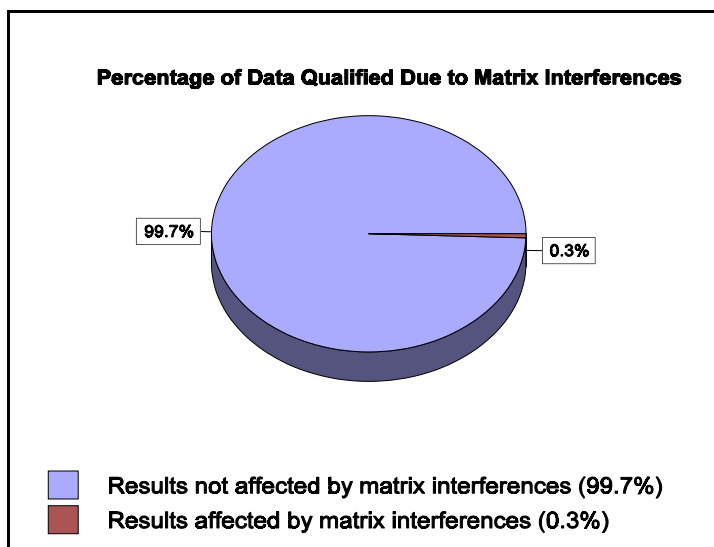
It is important to note that no blank subtraction was performed to eliminate the effects of blank contamination detected in the equipment, calibration, or method blank.<sup>2</sup> Instead, data associated with contaminated blanks were qualified so that data users could make decisions regarding data useability.

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<sup>2</sup> Mercury results were corrected for bubbler blank contamination as per sample calculation procedures described in Method 1631B. As with results from other analyses, however, none of the mercury results were corrected for contamination observed in the equipment, calibration, or method blanks.

#### 4.2.4 Spiked Sample Recoveries

All laboratories were required to spike field samples to estimate the recovery of target pollutants from the tissue samples analyzed in this study. The GC/MS methods used to analyze dioxins/furans, PCB congeners, and semivolatile organics required that isotopically labeled analogs of the target pollutants be spiked into each and every sample, including QC samples. This technique, known as isotope dilution, provides an extremely accurate means of quantifying a large number of pollutants in the presence of matrix interferences, and each method specifies acceptable recovery windows for the labeled compounds. Because the isotope dilution technique incorporates recovery-correction into calculations of target analyte concentration, any results that fail to meet the method-specified recovery windows are considered to be estimated values.



The gas chromatography (GC), atomic absorption (AA), and atomic fluorescence (AF) methods used to analyze pesticides, aroclors, arsenic species and mercury require that a matrix spike (MS) and a matrix spike duplicate (MSD) pair be prepared and analyzed with each batch of 20 field samples. QC samples are not subjected to the matrix spiking requirement. The methods provide precision and accuracy criteria that should be met for each analyte. Precision criteria are expressed as the relative percent difference (RPD) between the MS and MSD results, and accuracy criteria as expressed as acceptable recovery of each analyte. RPD is determined as:

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \quad \text{where, } C_1 \text{ is the concentration of the field sample and } C_2 \text{ is the concentration of its replicate.}$$

Unlike isotope dilution GC/MS techniques, the calculations involved in measuring pollutants by GC, AA, and AF techniques do not include a recovery-correction component. Therefore, the direction of the matrix spike recovery failure is used to estimate the directional bias of any associated sample results.

All labeled compound and MS/MSD results reported in Year 1 of the National Fish Tissue Study were carefully reviewed to evaluate the quality of data. In reviewing these results, reviewers verified that appropriate spiking compounds and spiking levels were used, that samples were spiked at the correct frequency, and that spiked sample results met method-specified criteria. In evaluating spiked sample results, data reviewers applied the following rules:

- If the isotopically labeled analog of a target compound was recovered in a sample above method-specified criteria, the associated native compound was coded with "HLBL" to indicate the presence of a high labeled compound recovery. The result also was qualified with an "Estimated

Value” descriptor to indicate that, although there was no question as to the identify of the pollutant, there is some doubt as to the reliability of the measured concentration. In Year 1, only two results received this qualifier.

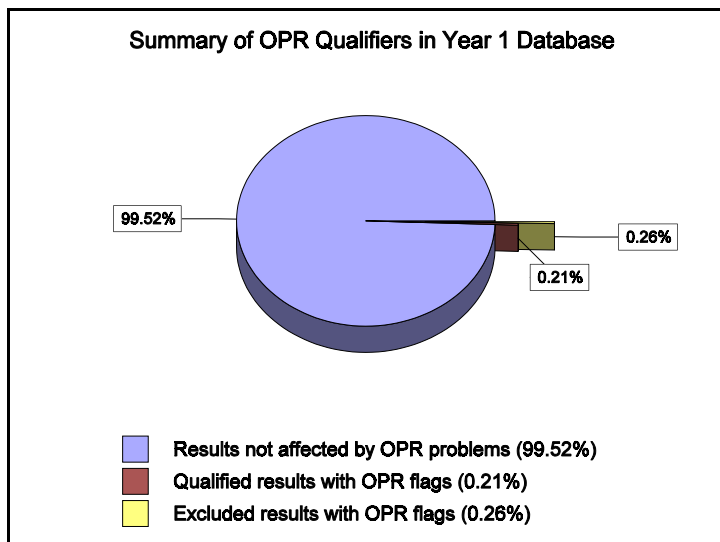
- If the isotopically labeled analog of a target compound was recovered below the method-specified criteria, the associated native compound in that sample was coded with “LLBL” to indicate the presence of a low labeled compound recovery. The result also was qualified with an “Estimated Value” descriptor. In Year 1, only three results received this qualifier.
- In rare cases, a labeled analog is not recovered from the sample at all. Due to the extreme nature of this QC failure, any associated native (i.e., target) compound result is considered unreliable and should, therefore, be excluded from the database. In Year 1, this situation occurred for only semivolatile organic analytes in 8 samples (a total of 10 results). Although relatively poor performance of these analytes (pentachlorophenol and 2,4-dinitrophenol) by Method 1625 was expected at the outset of the study, EPA decided to target the pollutants anyway as a means of obtaining as much useful data as possible without increasing analytical costs. The 10 affected Year 1 results were excluded from the database, and the associated sample records were coded as NLBL and further qualified with “Exclude” to explain the absence of these results.
- If an analyte was recovered from an MS or MSD sample above method-specified criteria, all associated samples with positive results were coded with “HMSR” to indicate the high matrix spike recovery. The associated samples also were qualified to indicate a “Potential High Bias” unless other flags (such as a “J”) applied to the same result suggested that the sample should be qualified as an “Estimated Value”. In Year 1, only 9 results were qualified with the HMSR flag (all 9 were for either arsenite or delta-BHC). Five of these results were reported below the quantitation limit and were qualified as estimated values; the remaining four results were qualified as having a “Potential High Bias”. Non-detects in samples associated with the high MS/MSD recovery were not flagged because it was clear that the potential high bias indicated by the MS or MSD had no adverse impact on the sample result.
- If an analyte was recovered from an MS or MSD sample below method-specified criteria, all associated samples were coded with “LMSR” to indicate the low matrix spike recovery. The associated samples also were qualified to indicate a “Potential Low Bias.” Nearly all of the LMSR codes applied during Year 1 of the study were for monomethylarsonic acid (MMA), suggesting that method improvements are needed to yield optimal results when determining this form of arsenic in tissue matrices.
- The first batch of samples analyzed for organophosphate pesticides by Method 1657A yielded no recoveries of Disulfoton in the MS and MSD samples analyzed with the batch and low recoveries of the same compound in the ongoing precision and recovery sample (OPR, see section 4.2.5) analyzed with the batch. Disulfoton was not reported in any of the samples nor could it be seen in a careful review of the sample chromatograms. Because it is impossible to determine if the non-detects reported in the samples are valid, results for all 18 samples were excluded from the database. These samples were flagged with “NMSR, LOPR” to indicate that there was no matrix spike recovery and low OPR recovery and further qualified with “EXCLUDE” to explain the absence of results. Data users should note, however, that this compound was not detected in any of the samples analyzed in Year 1, even when laboratory and method performance was in control.

#### 4.2.5 Ongoing Precision and Recovery

All laboratories that participated in the National Fish Tissue Study were required to prepare and analyze an ongoing precision and recovery, or OPR, standard with each sample set and to meet OPR acceptance criteria specified in the 1600 series method used to analyze the samples. The OPR standards are identical to those used in the IPR demonstrations discussed in Section 4.1.2.

A single OPR standard is analyzed with each sample batch to verify that laboratory performance is in control during the analysis of field samples. Whereas the calibration verification (discussed in Section 4.2.1) allows verification that the instrument remains in control during analysis of each batch, the OPR allows verification that the entire analytical process, including the instrumentation, is in control. Likewise, the OPR differs from the MS/MSD or labeled analog spikes described in section 4.2.4 in that the OPR is performed in a reference matrix to verify that all laboratory systems are in control, whereas the MS/MSD and labeled analogs are spiked into actual samples to verify that the method is working as expected in the actual matrices analyzed.

Each Year 1 data package generated in the National Fish Tissue Study was reviewed to verify that OPR samples were prepared and analyzed at the required frequency and that recoveries met acceptable performance criteria. All of the methods used in this study list OPR acceptance criteria that are applicable to fish tissue, except for Method 1625. Therefore, OPR data generated for Method 1625 were assessed against the OPR criteria specified in the method for aqueous samples. In evaluating OPR results, data reviewers applied the following rules:



- If the OPR results were only marginally outside the method criteria (i.e., results were within 10% of the method-specified windows), the data were considered to be acceptable without qualification. This is because all OPR windows established for the 1600 series methods used in this study reflect a 95% confidence interval. Data that are slightly outside this 95% confidence interval are considered sufficiently reliable to meet data quality objectives for the National Fish Tissue Study.
- If the OPR recovery was more than 10% lower than method-specified criteria, all tissue data associated with that OPR were qualified with either “LOPR” to indicate that a low OPR recovery of the target pollutant or “LLRO” to indicate a low labeled analog recovery from the OPR. Results with low target pollutant recoveries (i.e., LOPR results) were further qualified to indicate a “Potential Low Bias.” In Year 1, LOPR results were observed for only a handful of the pesticides measurements, most of which were associated with other data qualifiers (e.g. data also were reported below the ML). Likewise, only 2 semivolatile organic results were flagged with LLRO due to low labeled compound recovery in the OPR. One of these results was further qualified as an “Estimated Value”. The other result was excluded from the database as described in the next bullet.
- If the OPR recovery was more than 10% higher than method-specified criteria, all tissue data associated with that OPR were qualified. In Year 1, only one high OPR recovery was observed (for 2,6-dinitrotoluene in one sample). In this instance, the associated sample was flagged with “HNRO” to indicate high native compound recovery from the OPR. This result, however, was excluded from the database because the result also was associated with a low labeled compound

- recovery in the OPR and because the analytical holding time had been exceeded, raising significant questions about the reliability of the result.
- In rare cases, the labeled and/or the native compound was not recovered from the OPR sample, and associated results were excluded from the database. The associated sample records were coded as either “NLRO” or “NNRO” respectively, and further qualified with “Exclude” to explain the absence of any results. This situation occurred only for a handful of semivolatile organic measurements made by Method 1625. As was noted above in Section 4.2.4, relatively poor performance of these analytes was expected at the outset of the study, but the pollutants were targeted anyway as a means of obtaining as much useful data as possible without increasing analytical costs. EPA intends to continue this approach in Year 2 because useful results were obtained for many of the samples.

#### 4.2.6 *Holding Time Assessments*

Each data submission was reviewed to verify that all samples were received and maintained in frozen condition until analysis. When samples were thawed, the time between thawing and sample digestion, extraction, or analysis was assessed to verify that the holding times specified in each method were met. Samples that failed to meet the required holding times were coded with “HTEX” to indicate the holding time exceedance. If no other QC failures occurred, the data were coded to indicate a “Potential Low Bias.” In Year 1, such qualifiers were applied to only 11 results in the semivolatile organics data sets. Another 11 semivolatile organics results were analyzed outside holding time and were reported below the ML. These results were coded with both “HTEX” and “J”, and were further qualified as “Estimated Values.” One other result also was coded with an HTEX flag but this result was excluded for other reasons previously cited in Section 4.2.5 this report.

99.8% of the 100,018 sample results reported during Year 1 were determined within analytical holding times.
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#### 4.2.7 *Method Specific Considerations*

Methods 1656 and 1657 rely on the use of gas chromatography (GC) techniques coupled with selective detectors to determine organochlorine and organophosphate pesticides, respectively. The advantage of these techniques is that they allow for measurement of pesticides at lower concentrations than is possible with GC/MS. The disadvantage is that compound identification with the selective detectors is not as reliable as compound identification via GC/MS. To overcome this disadvantage, the methods require that any detected results be verified on a second column and that the results from the second column closely agree with the primary column results (the results measured on the two columns must agree within a factor of two). When such verification occurs, the laboratory reports the lower of the two results.

In reviewing each pesticides data package, reviewers evaluated the data to verify that all positive results were detected on two columns and that the results agreed within a factor of two. When the analyte was detected on both columns, but the results did not closely agree, the lower result was reported in the database and coded with “RNF2” to indicate that the results did not agree within a factor of two and further qualified as an “Estimated Value.” When the analyte was detected on one column but could not be confirmed on a second column, the result was coded “NCNF” to indicate the lack of confirmation and further qualified as an “Estimated Value. This approach was taken to advise data users about the possibility that an analyte might be present so that informed decisions can be made about the value of gathering additional data. For all pesticides except kepone, unconfirmed results were reported as non-detects unless confirmation on the second column was not possible due to co-elution. Because kepone

cannot be independently resolved on the second column, unconfirmed kepone results were reported as detects but qualified as described above. Several possible kepone hits are reported and flagged as NCNF in the database. In addition, one Year 1 result for trans-nonachlor could not be confirmed because the compound co-eluted with another pollutant on the second column. The co-eluted results also differed from the first column result by more than a factor of two. Therefore, this result was flagged with "NCNF, RNF2" and further coded as an estimated value.

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***NOTE: EPA reminds all data users that the identification of any pesticides qualified with "NCNF" or "RNF2" was not confirmed and appropriate caution should be exercised when using such results.***

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Finally, six pesticide results were coded "MTRX" to indicate that the sample chromatograms suggested the presence of possible matrix interferences. In three of these cases, the pesticide was detected on both columns, but the results did not agree within a factor of two, so the data were further coded with an "RNF2" flag. One of these three results also was reported below the ML on one of the columns so this result was further coded with a "J" flag. All six of these MTRX flagged results were qualified to indicate that they should be considered "Estimated Values."

### **4.3 Overall Data Quality Assessment**

Upon completion of all data review and database development activities for Year 1, the full data set was evaluated to determine if the results overall were falling within the measurement quality objectives (MQOs) established in the study QAPP.<sup>3</sup> Assessment of the data against these MQOs is described in Sections 4.3.1 through 4.3.5 below.

#### *4.3.1 Precision*

Precision is the degree of agreement among replicate measurements of the same property, under prescribed similar conditions [5 in the QAPP]. It can be expressed either as a range, a standard deviation, or a percentage of the mean of the measurements.

Ideally, precision is measured by subdividing samples in the field, preserving and numbering each split separately, and sending the aliquots to the analysis laboratory as "blind" duplicates. In this study, however, samples have to be homogenized, composited, and subdivided in a strictly controlled, clean laboratory environment. Therefore, the Sample Preparation Laboratory was required to prepare "duplicate composite pairs" on 5% of the samples analyzed. These duplicate composite pairs were sent to each analysis laboratory as "blind duplicates" (e.g., labeled with separate EPA sample numbers) and used to assess variability arising from the sample homogenization, compositing, aliquotting, shipping, and laboratory analysis processes.

Because agreement between results was expected to be better at higher concentrations, two MQOs were established. For sample results that were close to (i.e., less than 5 times) the ML, the MQO was that 90% of results from the original sample and the blind composite duplicates should agree within  $\pm 100\%$ . For

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<sup>3</sup>*Quality Assurance Project Plan for Analytical Control and Assessment Activities*, September 2000, Prepared for the US EPA Office of Water by DynCorp Environmental.

sample results that were well above (i.e., more than 5 times higher than) the ML, the MQO was that at least 90% of the results from the original and blind composite duplicate samples agree within  $\pm 50\%$ .

A total of 15 blind composite duplicates were prepared and analyzed for all of the target pollutants during Year 1. These analyses yielded approximately 5,000 pairs of detected results (i.e., the result was detected in both the field sample and its blind composite duplicate). Agreement between non-detects could not be quantitatively analyzed and is, therefore, not included in this discussion.) Approximately 1,100 of these paired results were detected at concentrations that were at least 5 times greater than the ML, and all (100%) of these results met the 50% MQO established for agreement between pollutants detected at this level. The remaining pairs were detected within 5 times the ML and subjected to the  $\pm 100\%$  objective described above. All but 3 of these pairs (99.8% of the results) met the MQO.

The study MQO for precision was exceeded during Year 1 of the study. One hundred percent (100%) of the duplicate composite pairs agreed within  $\pm 50\%$  when the measured results were more than 5 times the ML, and 99.8% of the duplicate composite pairs agreed within  $\pm 100\%$  when measured results were less than 5 times the ML.

In addition to the use of the “duplicate composite pairs” described above, the study design specifies that 10% of the lakes will be re-sampled. The relative percent difference (RPD) between these replicated samples provides information about the precision of data within the sub-sampled lakes and variability among the lakes. A total of 26 field replicates were collected and analyzed for all target pollutants in Year 1 of the study. These replicate pairs yielded 7,045 pairs of results with 'hits' detected at concentrations that were less than 5 times the ML. Of these paired results, 98.8% agreed within  $\pm 100\%$  of each other. A total of 1,639 of the field duplicate pairs yielded results that were more than 5 times greater than the ML; 922 (or 56%) of these results agreed within  $\pm 50\%$  and 1,113 (67.9%) of these results agreed within 100%. These figures suggest that overall agreement between the field samples and field duplicates is very good for results that are close to the ML, and fairly good for results reported at higher concentrations, despite the fact that each sample represents a group of completely different specimens.

It should be noted that nearly all of the wide RPD values observed between field samples and their field replicates were for PCB, dioxin, or furan congeners. Most likely this observation is attributable to the fact that the vast majority of pollutants detected in Year 1 of the study were for PCB, dioxin, and furan congeners. It is interesting to note, however, that although mercury was detected in nearly every sample, none of the wide RPDs observed in the Year 1 data set were for mercury. Further analysis of the sample results themselves may suggest that this is either an artifact of the distribution of mercury as compared to the distribution of other pollutants, or that it is due to the relatively high levels of mercury reported throughout the data set.

#### 4.3.2 Bias

Bias is the systematic distortion of a measurement process that causes errors in one direction.<sup>4</sup> In this study, bias from the analytical process was measured by preparing and analyzing field samples spiked with 1) the analytes of interest (i.e., matrix spike samples), 2) isotopically labeled analogs of the target pollutants, or 3) surrogate compounds that are expected to behave in a manner similar to the target

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<sup>4</sup>EPA Guidance for Quality Assurance Project Plans, EPA QA/G-5, EPA Office of Research and Development, Washington, D.C., 20460. EPA/600/R-98/018.



analytes. Assessment of these spiked sample results was described in Section 4.2.4. The MQO for overall analytical accuracy in this study was for 80% of these spiked field sample results to fall within the acceptance criteria specified for each method. This goal was easily exceeded in Year 1 of the study, suggesting that overall, the methods selected for use in the study are appropriate for the pollutants and matrices targeted.

The study MQO for bias was exceeded during Year 1 of the study; 99.7% of the spiked sample results fell within method-specified acceptance criteria.

Although the MQO was established for the entire data set, it is useful from a QA perspective to evaluate bias across individual methods to verify that each method is working as planned. On this basis, it is clear that the individual methods are working as intended. No matrix spike failures were observed for mercury or organophosphate pesticides, and 95% of the spiked organochlorine pesticide results met method-specified criteria. Most of the 5% that failed to meet the criteria were for Ethalfluralin, suggesting one area for further method improvement. Matrix spike samples performed for arsenic species also showed that, although the method works well in tissue for total inorganic arsenic and most of the other species measured, additional improvements are needed to yield optimal results for monomethylarsonic acid (MMA). If possible, these improvements will be implemented during the Year 2 analysis. Finally, all of the methods that involve spiking isotopically labeled compounds into each sample yielded a success rate of greater than 99.9%, with only 0.02%, 0.03%, and 0.04% of the labeled analog recoveries failing to meet method specified recoveries for dioxins/furans, PCBs, and semivolatile organics, respectively.

#### 4.3.3 Accuracy

Accuracy is a measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components that result from sampling and analytical operations. Accuracy is determined by analyzing a reference material of known pollutant concentrations or by reanalyzing a sample spiked with a known amount of pollutant.<sup>5</sup> Study objectives dictated that certified reference materials (CRMs) be sent to each laboratory to assess bias when available and feasible. No certified reference materials were sent during Year 1. These materials will be sent in Year 2 if available and if funding allows.

#### 4.3.4 Sensitivity

As described in Sections 4.1.1, all laboratories demonstrated their ability to achieve the study MQOs established for sensitivity, and 100% of the results generated during Year 1 of the study were generated on instruments that were calibrated to encompass minimum levels (ML) of quantitation. In addition, all results reported below the ML have been qualified in the database to alert data users that, although the presence of the pollutant was confirmed, the concentration reported in the database is an estimate because it falls below the quantitation limit.

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<sup>5</sup>EPA Guidance for Quality Assurance Project Plans, EPA QA/G-5, EPA Office of Research and Development, Washington, D.C., 20460. EPA/600/R-98/018.

#### 4.3.5 Completeness

Completeness is defined in terms of the percentage of data that are collected and deemed to be acceptable for use in the study. Three measures of completeness can be defined, as follows:

<b>Sampling Completeness:</b>	The number of valid samples collected relative to the number of samples planned for collection
<b>Analytical Completeness:</b>	The number of valid sample measurements relative to the number of valid samples collected; and
<b>Overall Completeness:</b>	The number of valid sample measurements relative to the number of samples planned for collection.

The analytical completeness goal in this study is that EPA obtain valid measurements from 90% of the valid samples collected. This goal was exceeded during Year 1 of the study. Only 2 samples collected in Year 1 were not analyzed at all, and a third was partially analyzed. One of the two not-analyzed samples was cancelled because it did not conform to design requirements. The other was a bottom feeder that had been inadvertently grouped with predators. The error was not identified until the batch was pulled for homogenization and compositing, and by this time, Sample Prep work for Year 1 bottom feeders had already been completed. The sample will be prepared with the first batch of Year 2 samples. A third sample was a predator consisting of such small fish that sufficient volume was unavailable to analyze for all target pollutants. Instead the sample was analyzed for metals and pesticides. The remaining volume was put on hold pending review of the corresponding bottom feeder results to determine analytical priorities for this sample.

Finally, readers are again cautioned that this QA report focuses on samples actually collected and analyzed during Year 1 rather than those statistically designated as Year 1 (i.e., Panel 1) samples. (See discussion in Chapter 1 for further details.) Due to this difference, EPA intends to wait until the study is completed to evaluate sampling and overall completeness measures.

EPA obtained valid measurements from 99% of the samples collected in Year 1 of the study, thereby exceeding the study MQO of 90% for analytical completeness during Year 1.
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**Exhibit 4-1. Summary of Data Review Qualifiers Used for Year 1 of the National Fish Tissue Study**

<b>Qualifiers Applied (SCC Code)</b>	<b>Full Length Description or Recommendation (Comment)</b>	<b>Explanation of Code (Reason)</b>	<b>Notes on When This Occurs</b>
B	Blank Contamination	The target analyte was detected in one or more of the blanks associated with this sample.	Applied to fewer than 0.007% of Year 1 results and only occurs for PCB congener totals in which some of the individual totals were associated with B flags.
B, J	Blank Contamination, Estimated Value	<ul style="list-style-type: none"> <li>One or more of the PCB congeners contributing to the total was associated with a contaminated blank <i>and</i> one or more of the congeners was reported above the MDL and below the ML. In most cases, the impact of the flag on the total is negligible due to the relatively minor impact of the individual congener(s) the flags represent.</li> <li>The dioxin sample result was reported above the MDL but below the ML and is, therefore, an estimated value. The result also was associated with a contaminated blank that was at least 5 times less than the reported result.</li> </ul>	<p>Applied to 1% of Year 1 results, but only for PCBs and dioxins.</p> <ul style="list-style-type: none"> <li>In the PCB database, this combination only occurs for PCB congener totals in which flagged B and flagged J results from individual congeners are mathematically summed with unflagged results.</li> <li>Applies only to a handful of individual congener results in the dioxin database and occurs only because the dioxin MDLs were more than 10 times lower than the MLs at which the instruments were calibrated.</li> </ul>
B, RMAX	Blank contamination, result is a maximum value	Blank contamination was observed but the target analyte was reported in the sample at a concentration between 5 and 10 times higher than the blank value. The result was considered to be of acceptable quality but data users are cautioned that it may be a maximum value due to possible influence of contamination.	Applied to 0.18% of Year 1 results, and only for a handful of PCB congener and dioxins/furans.
B, RNAF	Blank Contamination, Result Not Affected	Blank contamination was present but was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples.	Applied to 1.3% of the Year 1 results. Occurs in all data sets except metals.

<b>Qualifiers Applied (SCC Code)</b>	<b>Full Length Description or Recommendation (Comment)</b>	<b>Explanation of Code (Reason)</b>	<b>Notes on When This Occurs</b>
B, RNON	Blank contamination, result a non-detect	When the sample result is less than five times the blank result, there are no means by which to ascertain whether or not the presence of the analyte may be attributed to contamination. Therefore, SCC recommends that the data be reported in the database as a non-detect at the ML, adjusted for dilution.	Applied to 1% of Year 1 results, with most of the occurrences for non-toxic PCB congeners.
HLBL	Estimated Value	The labeled analog of the target compound was recovered above method-specified criteria suggesting a possible matrix interference (High Labeled Compound Recovery).	Applied to two results in the PCB data set.
HMSR	Potential High Bias	High analyte recovery was observed in one or more matrix spike samples associated with this result.	Applied to two arsenite results and two delta-BHC results.
HNRO, HTEX, LLRO	Exclude	A combination of QC failures that suggest the reliability of the result is questionable. In this case, the native compound was recovered above method-specified criteria and the labeled analog was recovered below method-specified criteria in the OPR associated with sample (HNRO and LLRO). In addition, the holding time was exceeded (HTEX) and, although the compound was reported in a sample dilution, it was not found in the neat analysis.	Applied to one 2,6-dinitrotoluene result.
HSSR	Potential High Bias	A high surrogate spike recovery was observed suggesting a possible high bias in the result reported.	Occurred for one 4,4'-DDE result.
HTEX	Potential Low Bias	Holding Time Exceeded.	Associated only with five n-Hexadecane and six n-Octadecane results.
HTEX, J	Estimated Value	The sample result reported was above the MDL (detection limit) but below the ML (quantitation limit) and the holding time was exceeded when analyzing the sample.	Associated only with nine n-hexadecane, one bis(2-ethylhexylphthalate), and one n-octadecane result.

Qualifiers Applied (SCC Code)	Full Length Description or Recommendation (Comment)	Explanation of Code (Reason)	Notes on When This Occurs
HVER, J, RNF2	Estimated Value	The sample result was determined on both pesticide columns, but the lower of the two results reported was below the quantitation limit, <i>and</i> the results reported were not within a factor of two of each other (possibly due to the low level reported.) In addition, the lower of the two values was associated with a high calibration verification standard recovery.	Occurred for one 4,4'-DDT result.
HVER, RNF2	Estimated Value	The sample result was determined on both pesticide columns, but the results reported were not within a factor of two of each other <i>and</i> the lower of the two values was associated with a high calibration verification standard recovery.	Occurred for two methoxychlor results.
J	Estimated Value	The sample result reported was above the MDL (detection limit) but below the ML (quantitation limit).	Occurred throughout all the data sets.
J, HMSR	Estimated Value	The sample result reported was above the MDL but below the ML <i>and</i> a high recovery of this result was observed in an associated matrix spike sample suggesting the J-flagged result may have a high bias.	Applied to three arsenite results and two delta-BHC results.
J, HSSR	Estimated Value	The sample result was reported above the MDL but below the ML on at least one of the two columns on which the pesticide was detected <i>and</i> a high recovery of the surrogate spike compound was associated with the reported result.	Applied to one alpha-chlordane result.
J, LLBL	Estimated Value	The target analyte was reported above the MDL but below the ML <i>and</i> the labeled analog of the target was recovered below method-specified criteria suggesting the possible presence of matrix interferences.	Applied to one hexa-dioxin result and one n-tetracosane result.
J, LLRO	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample <i>and</i> the labeled analog of the target analyte was recovered below method criteria in the OPR associated with the sample.	Applied to one 2,4-dimethylphenol result.

Qualifiers Applied (SCC Code)	Full Length Description or Recommendation (Comment)	Explanation of Code (Reason)	Notes on When This Occurs
J, LOPR	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML <i>and</i> the target analyte was recovered below method-specified criteria in the OPR associated with the sample.	Applied to two 4,4'-DDT results.
J, LOPR, NCNF	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML <i>and</i> the target analyte was recovered below method-specified criteria in the OPR associated with the sample <i>and</i> the result was not confirmed on a second column.	Applied to six kepone results.
J, LOPR, RNF2	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML <i>and</i> the target analyte was recovered below method-specified criteria in the OPR associated with the sample <i>and</i> , although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to two 4,4'-DDT results.
J, MTRX, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML <i>and</i> the chromatogram suggested possible matrix interferences with this sample <i>and</i> , although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one cis-permethrin result.
J, NCNF	Estimated Value	The target analyte was reported above the MDL but below the ML <i>and</i> the result was not confirmed on a second column.	Applied to a handful of pesticide results.
J, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML <i>and</i> , although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to a handful of pesticides results.
LLBL	Estimated Value	The labeled analog of the target analyte was recovered below method-specified criteria.	Applied to one n-hexadecane result.

Qualifiers Applied (SCC Code)	Full Length Description or Recommendation (Comment)	Explanation of Code (Reason)	Notes on When This Occurs
LMSR	Potential Low Bias	Low analyte recovery was observed with one or more matrix spike samples associated with this result.	Applied primarily to monomethylarsonic acid and to a small number of total inorganic arsenic results. Also applied to one Endosulfan sulfate result.
LMSR, LOPR	Potential Low Bias	Low analyte recovery was observed with one or more matrix spike samples <i>and</i> with the OPR sample associated with this result.	Applied to a few Endosulfan II and Endosulfan sulfate results.
LOPR	Potential Low Bias	Low analyte recovery was observed with the OPR sample associated with this result.	Applied to several results for kepone, 4,4'-DDT, Endosulfan sulfate, and Ethalfluralin
LOPR, NCNF	Estimated Value	Low analyte recovery was observed with the OPR sample associated with this result <i>and</i> the presence of the reported analyte was not confirmed on a second column.	Applied to four kepone results (kepone cannot be confirmed on the method-specified second column).
LOPR, NMSR	Exclude	Low analyte recovery was observed with the OPR sample associated with this result <i>and</i> the analyte was not recovered at all in the matrix spike sample associated with the result. The pollutant was not detected in the sample but the recovery problems observed in the OPR and the matrix spikes make it impossible to confirm the reliability of these non-detects.	Applied to 18 disulfoton results.
LOPR, RNF2	Estimated Value	The pollutant was detected on both columns, but the results were not within a factor of two of each other <i>and</i> the lower of the results reported was associated with a low OPR standard recovery.	Applied to one Ethalfluralin result.
LVER	Potential Low Bias	Low analyte recovery was observed in a calibration verification associated with this sample, suggesting the possibility of a low bias in the result.	Applied to two 4,4'-DDT results.
LVER, RNF2	Estimated Value	The pollutant was detected on both columns, but the results on the two columns differed by more than a factor of two, <i>and</i> the lower of the two results reported was associated with a low recovery of calibration verification standard.	Applied to one 4,4'-DDT result.

Qualifiers Applied (SCC Code)	Full Length Description or Recommendation (Comment)	Explanation of Code (Reason)	Notes on When This Occurs
MTRX	Estimated Value	The chromatogram suggested possible matrix interferences with the sample.	Applied to one methoxychlor and two cis-permethrin results.
MTRX, RNF2	Estimated Value	The chromatogram suggested possible matrix interferences, <i>and</i> although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one methoxychlor and one cis-permethrin result.
NCNF	Estimated Value	The result was not confirmed (either no elution or co-elution on a second column).	Applied to 20 kepone results (kepone cannot be confirmed on the method-specified second column).
NCNF, RNF2	Estimated Value	The result was found on one column but co-eluted with another compound on the second column. The co-eluted results differed from the other column results by a factor greater than two.	Applied to one trans-nonachlor result.
NLBL	Exclude	The labeled analog was not recovered from the sample suggesting severe matrix interferences. Because it is impossible to determine if the analyte is present or not present, the reported target analyte result was excluded from the database.	Applied to four pentachlorophenol results.
NLBL, NLRO, NNRO	Exclude	The labeled analog was not recovered from the sample nor from the OPR and the target analyte was not recovered from the OPR. The failures suggest poor method performance for this analyte.	Applied to six 2,4-dinitrophenol results.
NLRO, NNRO	Exclude	Neither the target analyte (native compound) nor the labeled analog were recovered in the OPR sample associated with this result.	Applied to a handful of semivolatile organics pollutant results.
NNRO	Exclude	The native compound was not recovered in the OPR sample suggesting that the method may not be working for this analyte.	Applied in the semivolatile organics database. Mostly applied to hexachlorocyclopentadiene, but also applied to a handful of other results in this database.



Qualifiers Applied (SCC Code)	Full Length Description or Recommendation (Comment)	Explanation of Code (Reason)	Notes on When This Occurs
REXC	Estimated Value	The result exceeded the instrument calibration range.	Applied to one di-n-octyl phthalate result.
RNF2	Estimated Value	Although the analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to a handful of organochlorine pesticide results.